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CONFERENCE

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TITLE (QPS 2010-005)

Method Development and Validation of Qualitative ELISAs in a Multi-Tiered Approach to Immunogenicity Testing of a Pegylated Therapeutic Protein

AUTHORS

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ABSTRACT

Introduction

To develop and validate ELISAs, which are specific for both antigen epitopes and antibody isotypes to be used in a multi-tiered approach for detection of human anti-drug antibodies (ADA) against a pegylated protein therapeutic.

Methods

The protein therapeutic drug is a pegylated protein. Due to the multimeric structure of this molecule, a direct ELISA design was adopted. Antigen was coated on the microtiter plate, and ADA were detected with anti-species (human and rabbit) antibody.

Affinity purified rabbit anti-drug (PCD), anti- protein (PCB) and monoclonal anti-PEG (PCP, Epitomics) were used as positive controls (PCs). Because there was no cross-reactivity between PCD and PCB, pegylated protein (drug) and unpegylated protein were coated separately. Goat anti-human IgG and IgM antibodies were used in different assays for antibody subtype testing. In total, four ELISAs were developed to detect anti-drug and anti-protein IgG and IgM antibodies.

In each assay, screening and confirmation cut points were determined by analyzing 51 individual normal human sera, with or without added drug, PEG or unpegylated protein. Assay precision was evaluated using PCs. Sensitivity was obtained by interpolating antibody concentration at the cut point from a curve of serially diluted rabbit PCwith results adjusted for differences due to anti-species (rabbit vs. human) conjugates. Assay free-drug tolerance and titration characteristics of positive controls were evaluated.

Results

Assay screening and confirmation cut points for anti-drug, anti-PEG and anti-protein were determined using robust statistical methods. All assays displayed acceptable precision. The data support assigning a unique floating cut point value for each plate by multiplying the negative control mean value by a constant factor. Assay sensitivity, drug tolerance and titration tests met criteria set forth in the validation protocol.

Conclusion

Four qualitative direct ELISAs were developed and validated to detect anti-drug, anti-PEG and anti-protein IgG and IgM antibodies to a pegylated protein drug. These validated assay parameters and acceptance criteria were used for clinical sample immunogenicity testing.